ORIGINAL ARTICLE

Solid Lipid Nanoparticles Incorporated Transdermal Patch for Improving the Permeation of Piroxicam

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Abstract

Aim: Piroxicam is class II drug and has low oral bioavailability owing to low aqueous solubility. Long-term administration of piroxicam is reported to produce gastrointestinal toxicity. The objective of this study was to improve the permeation of piroxicam by incorporating as piroxicam loaded solid lipid nanoparticles (pirox-SLNs) into a transdermal patch. **Method:** Pirox-SLN's (average particle size 248.87 ± 6.481 nm and entrapment efficiency 84.48% ± 1.08%) upon optimization, were prepared by pre-emulsion sonication method and were incorporated into ethyl cellulose and polyvinyl pyrrolidone matrix patch prepared by solvent evaporation method. **Results& Discussion:** The prepared transdermal patches were evaluated for thickness, weight variation, flatness, folding endurance, and drug content which were found to 0.31 ± 0.04 mm, 0.17 ± 0.03 g, 99.5% ± 0.3%, 35 ± 1.34 and 95.74 ± 0.4, respectively. *Ex-vivo* skin permeation of the prepared formulation was studied on rat skin and the drug release from patch incorporated with SLNs was found 66.6% up to 24 h, significantly less as compared to plain piroxicam patch having release of 88.01%, however, the slow release from the SLN patch was attributed due to slow release of the drug from SLN. *Ex-vivo* skin permeation studies on pirox-SLN containing patches showed satisfactory flux (17.16 μg/cm^2/h) compared with that of plain piroxicam patches (4.6 μg/cm^2/h). The skin irritation test showed that the prepared transdermal patch were free of skin irritants. **Conclusion:** It was concluded that SLN's can be successfully used as a carrier for enhancing transdermal permeation of piroxicam and thus the bioavailability.

Key words: *Ex-vivo* skin permeation study, piroxicam, skin irritation test, solid lipid nanoparticle

INTRODUCTION

A transdermal patch is used for delivery of medications through the skin for treating systemic illnesses.[1] Transdermal drug delivery systems, also known as "patches" offers a variety of benefits such as controlled release, reduced systemic side effects, user-friendly, painless, and patient compliance through multi-day dosing.[2] The transdermal route vies with the oral treatment as a most successful innovative research area in drug delivery platform.[3] Human skin mainly the stratum corneum presents as an efficient barrier to prevent the entry of foreign substances which is the main difficulty in achieving transport of therapeutic agents across.[4] The stratum corneum is effectively a 10-15 μm thick matrix of dehydrated, dead keratinocytes (corneocytes) embedded in a lipid matrix. Different approaches of penetration enhancement have been used so far to improve permeation of drugs through the skin. The widely used approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters) but is fraught with the problem of skin irritation and toxicity.[5] Newer approaches include the use of carriers/vesicles such as liposomes, niosomes, ethosomes, microemulsions, and complexes, which provide a good alternative technique to enhance permeation of drugs across stratum corneum. Enhancement of transdermal drug permeation of theophylline using microemulsion,[6] ketorolac using proniosomes[7] and ketotifen using ethosomes[8] have been reported. Solid lipid nanoparticles (SLNs) have also been investigated as carriers for enhanced skin delivery of Vitamins A and E.[9] Permeation enhancement is primarily due to small size and swelling of stratum corneum by an increase in skin hydration caused by the occlusive film of SLN.

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Piroxicam, a biopharmaceutical classification system Class II non-steroidal anti-inflammatory drug belonging to the oxicamcategory, has been used in conditions such as rheumatoid arthritis, osteoarthritis, and traumatic contusions. Piroxicam demonstrates slow and gradual absorption on oral administration has a long elimination half-life (37.5 h) and t_{max} of 3-5 h. Piroxicam is associated with numerous side effects including ulceration and gastric bleeding which limits its use thus, triggering need for an alternative non-invasive delivery method. Attempts to improve the transdermal availability of piroxicam have been reported using beta cyclodextrins as a carrier.[10] The objective of present study was to evaluate the use of piroxicam loaded SLN (Pirox-SLN) to improve permeation, sustained release, and bioavailability on the transdermal application in the form of the patch. It is also believed that transdermal delivery of Pirox-SLN can overcome the above-mentioned limitations of the oral use of piroxicam.

MATERIALS AND METHODS

Materials

Piroxicam was received as a kind gift sample from Wockhardt Pharmaceuticals R&D, Aurangabad, India. Compritol 888 ATO was received as a gift from Colorcon Asia Ltd., Goa, and all other chemicals were procured from local sources and were of analytical grade.

Methods

Preparation and evaluation of SLNs

Pirox-SLN dispersion was prepared using pre-emulsion probe sonication method. The optimization studies were premeditated using Box-Behnken design keeping drug to lipid ratio, concentration of lipid-based surfactant and sonication time as dependent variables and % entrapment and particle size as responses. For the preparation of SLN, the lipid, i.e., compritol ATO 188 was heated to 90°C, and piroxicam was dissolved in the lipid melt by stirring until the melt appeared clear (drug: lipid ratio, 1:8). An aqueous phase was prepared by dissolving polysorbate 80 (2% w/v) in distilled water. The hot aqueous phase was added to the oil phase and homogenized for 15 min using homogenizer (Omni international) at 35,000 rpm and temperature 70°C. The coarse hot oil/water emulsion so obtained was ultrasonicated using probe sonicator (Sonics Vibra cell VCX 750). Pirox-SLNs were obtained by allowing hot nanoemulsion to cool to room temperature.[11] The prepared SLN was evaluated for size using particle size analyzer (Malvern Master Sizer SM2000K) and entrapment efficiency by dissolving in an appropriate solvent and determining content by an ultraviolet spectrophotometer (Jasco V-530) at 355 nm.

Preparation of transdermal patches

Transdermal patches containing plain piroxicam and pirox-SLN were prepared as per Table 1.

Polymers were dissolved in chloroform/ethanol to which 40 mg piroxicam and 150 mg of plasticizer (dibutyl phthalate) were added. The resultant dispersion was stirred at 100 rpm for 60 min. The dispersion was then poured in petri-dish placed on the even surface and was allowed to air dry overnight. Circular patches of 2 cm diameter (3.14 cm^2) were cut from dried patches and placed in desiccators.[12]

Evaluation of transdermal patches

Thickness[13]

Thickness of the patches was measured using Digital Vernier calipers at three different places, and mean value was calculated.

Weight variation[14]

Weight variation was studied by individually weighing 3 randomly selected patches. Such determination was performed for each formulation and mean value was calculated.

Flatness[15]

Three longitudinal strips were cut out from each patch: 1 each from the center, left side, and right side. The length of each strip was measured, and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness.

$$\text{Constriction (\%)} = (L_1 - L_2)/L_2 \times 100$$

Where, L_1 initial length of each strip, L_2, final length.

Folding endurance[15]

Folding endurance was determined by repeatedly folding the patches at the same place until it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value.

Drug content[16]

A prepared patch was added to 100-mL saline phosphate buffer (pH 7.4) and stirred vigorously for 24 h followed by ultrasonication for 15 min. The contents were filtered, and piroxicam was estimated spectrophotometrically at wavelength of 355 nm.

In vitro drug release study[16,17]

In vitro drug release studies were performed by a Franz diffusion cell with a receptor compartment capacity of 7 ml. Cellophane membrane having pore size 0.45 µm and dialysis membrane having pore size 2.4 nm was employed for the determination of drug from plain Piroxicam and Pirox-SLN transdermal films, respectively. The receptor compartment of the diffusion cell was filled with saline phosphate buffer pH 7.4. The whole assembly was fixed on the three station diffusion cell apparatus, and the solution in the receptor compartment was constantly and continuously stirred at 600 rpm using magnetic beads and the temperature was

Table 1: Composition of transdermal patches

Formulation code	Therapeutic agent	Polymer ratio	Plasticizer (dibutyl phthalate) (mg)	Solvent (ml)	
		PVP: EC (total weight 500 mg)		Chloroform	Ethanol
F-1	Plain piroxicam 40 mg	2:3	150	10	-
F-2	Plain piroxicam 40 mg	3:2	150	10	-
F-3	Plain piroxicam 40 mg	1:4	150	10	-
F-4	Pirox-SLN equivalent to 40 mg of piroxicam	3:2	150	-	10

EC: Ethyl cellulose, PVP: Polyvinyl pyrrolidone, SLN: Solid lipid nanoparticles

maintained at 32 ± 0.5°C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically.

Ex-vivo skin permeation study[18]

Ex-vivo skin permeation studies were performed by the Franz diffusion cell with a receptor compartment capacity of 7 ml and effective diffusion area 3.14 cm^2. The excised rat (Wistar albino rat) abdominal skin was mounted between the donor and receptor compartment of the diffusion cell. The formulated patches were placed over the skin. The receptor compartment of the diffusion cell was filled with saline phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 600 rpm; the temperature was maintained at 32 ± 0.5°C. The samples were withdrawn at pre-determined time intervals (2, 4, 6, 8, 16, and 24 h) and analyzed for drug content spectrophotometrically at 355 nm. The cumulative amounts of drug permeated per square centimeter of patches were plotted against time.

Primary skin irritation study[19]

The hair on the dorsal side of Wistar albino rats was removed by clipping 1 day prior to this portion of the experiment. Rats were divided into three groups ($n = 6$). Group I served as the control, Group II received plain piroxicam containing patch, and Group III received pirox-SLN containing the patch. After 24 h, application site was graded according to visual erythema scoring method.

In-vivo kinetic study

The research protocol for *in-vivo* study was approved by the Institutional Animal Ethics Committee of AISSMS College of Pharmacy, Pune, India. Two groups of six male rats each weighing 200-250 g were used for each formulation. Of the two groups, the first group was meant for suspension of marketed piroxicam capsule, the second was meant for Pirox-SLN patch (F-4). About 2 ml of blood samples were withdrawn from retro-orbital plexus at 0, 2, 4, 6, 18, and 24 h. Blood samples were centrifuged at 3,000 rpm for 30 min, and plasma samples were separated. Plasma (0.5 ml) was transferred in a test tube to which 1.5 ml of methanol was added and vortex-mixed for 5 min to extract out the drug. The tube was centrifuged for 30 min at 3000 rpm, and the supernatant was subjected to the high-performance liquid chromatography analysis.[20]

RESULT AND DISCUSSION

Evaluation of the prepared SLNs

The prepared SLNs had an average particle size of 248.87 ± 6.481 nm and percent entrapment efficiency of 84.48 ± 1.08%. The probe sonication produces shock waves minimizing the globule size into nano range which turns into nanoscaled particles when cooled to room temperature.[21]

Preparation of transdermal patches

The patches with the plain drug were prepared with chloroform while the patch with SLN was prepared with ethanol so as to avoid solubilization of the SLN.

Evaluation of physio-chemical properties of transdermal patches

The transdermal patches containing plain piroxicam and pirox-SLN were prepared as per Table 1 and were subjected to evaluation the results of which is summarized in Table 2. The thickness of transdermal patches of ranged between (0.29 ± 0.01 to 0.31 ± 0.03 mm). The weight variation range of 0.12 ± 0.025 to 0.17 ± 0.03 g indicated that different batches of prepared patches had similar weights. The % flatness study showed values ranging between 99.18 ± 0.3 and 99.84 ± 0.4%, thus indicating a lack of constriction. Folding endurance results ranged between 33 ± 0.94 and 58 ± 0.66 indicating the capacity of patches to maintain their integrity with general skin folding when applied.

In vitro drug release study

For any drug to absorb across a biological membrane, it should release quickly and completely from the dosage form. The cumulative percentage release of piroxicam from prepared transdermal films was investigated for 24 h [Figure 1]. Percent drug release at the end of 24 h for formulation F-3

Table 2: Physiochemical properties of transdermal patches (data shows mean [*n*=3]±SD)

Parameter	Formulations code			
	F-1	F-2	F-3	F-4
Thickness (mm)	0.29±0.01	0.30±0.03	0.29±0.02	0.31±0.04
%Weight variation (g)	0.13±0.02	0.12±0.025	0.19±0.01	0.17±0.03
(%) Flatness	99.39±0.5	99.84±0.4	99.18±0.3	99.5±0.3
Folding endurance	38±2.2	58±0.66	33±0.94	35±1.34
Drug content (%)	97.32±0.3	98.61±0.2	96.69±0.5	95.74±0.4

SD: Standard deviation

was found to be 87.99% which is higher as compared to formulation F-1 and F-2. This can be explained by virtue of the higher content of hydrophilic polymer, i.e., polyvinyl pyrrolidone. The formulation F-4, which contained pirox-SLN in same polymer composition as that of formulation F-3exhibited 66% drug release which is understandable due to release retarding effect of the lipid nanoparticles.

Ex-vivo skin permeation study

The *ex-vivo* skin permeation of plain piroxicam transdermal patches and transdermal patches (formulation F-3 and F-4) was investigated through the rat abdominal skin as shown in Figure 2. The cumulative amount of drug permeated at the end of 24 h was found to be (411.69 ± 2.15 µg/cm^2) for transdermal patches which was significantly ($P < 0.05$) higher compared to (105.58 ± 1.5 µg/cm^2) for plain piroxicam patches. Results of the permeation parameters, such as steady state flux (Jss), lag time (Lt), permeability coefficient (P), and enhancement ratio (E), are as shown in Table 3.

The flux from formulation F-3 and formulation F-4 was found to be 4.6 µg/cm^2/h and 17.16 µg/cm^2/h, respectively. The required flux from transdermal patch can be calculated from the ratio of the product of total body clearance and average plasma concentration to the area of the patch.

$$\text{Flux (Jss)} = \text{CLt} * \text{CPss/A}$$

Where, CLt = Total body clearance, Cpss = Average plasma concentration, A = Surface area (A cm^2). Targeted flux of 16.1 µg/cm^2/h which was achieved with patches. The increase in permeability can be explained by nanosize of SLN particles and occlusion effect of SLN.

Skin irritation testing

Skin irritation testing of piroxicam transdermal patch showed skin irritation score (erythema) of <2 [Table 4].

According to Draize and Woodward, compounds producing scores of 2 or less are considered negative (no skin irritation). Hence, the developed transdermal formulations were free of skin irritation.

Table 3: Flux, lag time, permeability coefficient, and enhancement ratio of piroxicam patches and pirox-SLN patches

Permeation parameter	Formulation	
	Plain piroxicam patch	Pirox-SLN patch
Flux (µg/h/cm^2)	4.6	17.16
Lt (h)	4.0	6.0
Permeability coefficient (cm/h^{-1}×10^{-2})	0.216	0.5
Enhancement factor	-	3.73

SLN: Solid lipid nanoparticles

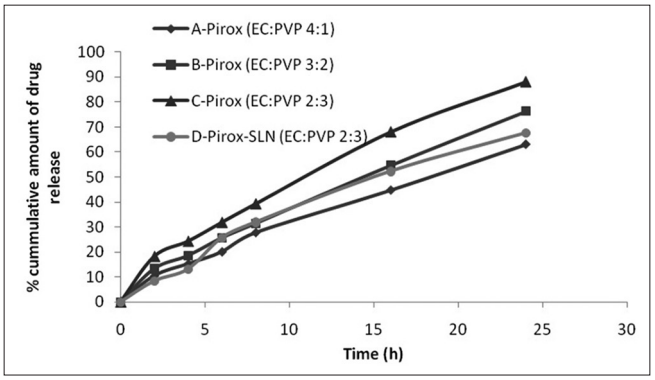

Figure 1: *In vitro* drug release study from transdermal patches

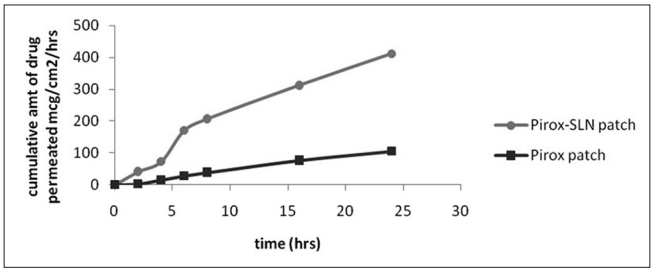

Figure 2: *Ex-vivo* skin permeation of piroxicam from piroxicam transdermal patch and pirox- solid lipid nanoparticles transdermal patch through rat abdominal skin

In-vivo kinetic study

The *in-vivo* study was performed to quantify piroxicam after oral and transdermal administration. Figure 3 shows

blood plasma levels piroxicam after oral and transdermal administration.

It was seen from Figure 3 that the plasma concentration profile of piroxicam for Pirox-SLN transdermal patches (F-4) showed greater improvement of drug absorption than the oral formulation. The mean pharmacokinetic parameters were calculated from the blood plasma concentration of the drug are shown in Table 5. The increase in the area under the curve (AUC) and C_{max} may be due to the enhanced skin permeation and improved solubility due to reducing the particle size of piroxicam.

Table 4: Skin irritation studies

Rat no	Control	Piroxicam patches	Pirox-SLN patches
	Erythema	Erythema	Erythema
1	0	1	0
2	0	1	0
3	0	1	0
4	0	0	0
5	0	1	1
6	0	0	0
Average	0	0.66±0.21	0.16±0.16

Data shows mean (*n*=6)±SE erythema scale: 0 - No erythema; 1 - Slight erythema (barely perceptible, pink), 2 - Moderate erythema (dark pink), 3 - Moderate to severe erythema (light red), 4 - Severe erythema (extreme redness), SLN: Solid lipid nanoparticles

Table 5: Mean pharmacokinetic parameter of piroxicam after oral and transdermal administration

Parameters	Oral formulation	Transdermal patches (F-4)
C_{max} (ng/ml)	544	556
t_{max} (h)	2.0	6.0
$AUC_{0\rightarrow24}$ ng h/ml	4617.6	10604.6

AUC: Area under the curve

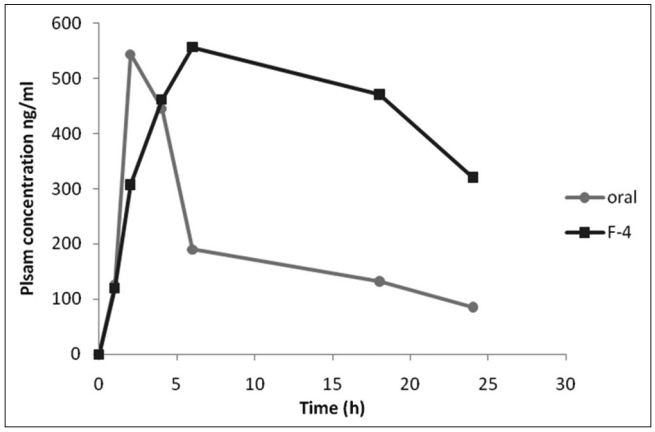

Figure 3: Plasma concentration profile of piroxicam after oral and transdermal administration

CONCLUSION

The prepared patches possessed satisfactory physiochemical characteristics. *Ex-vivo* skin permeation studies showed a drug flux of 17.16 μg/cm²/h from pirox-SLN patch compared to 4.6 μg/cm²/h from plain piroxicam patch attributing improved delivery of piroxicam from the transdermal patch. The increased AUC and C_{max} from *in-vivo* kinetic study further conclude that incorporation of drugs in the form of SLN can improve transdermal bioavailability.

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